

What is claimed:

1. An albumin fusion protein comprising a member selected from the group consisting of:

(a) a Therapeutic protein:X and albumin comprising the amino acid
5 sequence of SEQ ID NO:18;

(b) a Therapeutic protein:X and a fragment or a variant of the amino acid
sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity;

(c) a Therapeutic protein:X and a fragment or a variant of the amino acid
sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity, and

10 further wherein said albumin activity is the ability to prolong the shelf life of the
Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused
state;

(d) a Therapeutic protein:X and a fragment or a variant of the amino acid
sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity, and

15 further wherein the fragment or variant comprises the amino acid sequence of amino acids 1-
387 of SEQ ID NO:18;

(e) a fragment or variant of a Therapeutic protein:X and albumin
comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has
a biological activity of the Therapeutic protein:X;

20 (f) a Therapeutic protein:X, or fragment or variant thereof, and albumin,
or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment
or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment
or variant of albumin;

(g) a Therapeutic protein:X, or fragment or variant thereof, and albumin,
25 or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment
or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment
or variant of albumin;

(h) a Therapeutic protein:X, or fragment or variant thereof, and albumin,

or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin;

(i) a Therapeutic protein:X, or fragment or variant thereof, and albumin,
5 or fragment or variant thereof, of (a) to (e), which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof;

(j) a Therapeutic protein:X, or fragment or variant thereof, and albumin,
10 or fragment or variant thereof, of (a) to (i), wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker; and

(k) a Therapeutic protein:X, or fragment or variant thereof, and albumin,
15 or fragment or variant thereof, of (a) to (j), wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

and further wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or a fragment or variant of albumin.

20 2. The albumin fusion protein of claim 1, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

25 3. The albumin fusion protein of claim 1, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

4. The albumin fusion protein of claim 1, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

5. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin, or fragment or variant thereof, comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

6. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin, or fragment or variant thereof, comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 54 to 61 of SEQ ID NO:18;
- (b) amino acids 76 to 89 of SEQ ID NO:18;
- (c) amino acids 92 to 100 of SEQ ID NO:18;
- (d) amino acids 170 to 176 of SEQ ID NO:18;
- (e) amino acids 247 to 252 of SEQ ID NO:18;
- (f) amino acids 266 to 277 of SEQ ID NO:18;
- (g) amino acids 280 to 288 of SEQ ID NO:18;
- (h) amino acids 362 to 368 of SEQ ID NO:18;
- (i) amino acids 439 to 447 of SEQ ID NO:18;
- (j) amino acids 462 to 475 of SEQ ID NO:18;
- (k) amino acids 478 to 486 of SEQ ID NO:18; and
- (l) amino acids 560 to 566 of SEQ ID NO:18.

7. The albumin fusion protein of claim 5, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic

protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

8. The albumin fusion protein of claim 6, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

9. The albumin fusion protein of claim 5, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

10. The albumin fusion protein of claim 6, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

11. The albumin fusion protein of claim 5 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

12. The albumin fusion protein of claim 6 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the

Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

5 13. The albumin fusion protein of any one of claims 1-12, which is non-glycosylated.

 14. The albumin fusion protein of any one of claims 1-12, which is expressed in yeast.

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 15. The albumin fusion protein of claim 14, wherein the yeast is glycosylation deficient.

 16. The albumin fusion protein of claim 14 wherein the yeast is glycosylation and protease deficient.

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 17. The albumin fusion protein of any one of claims 1-12, which is expressed by a mammalian cell.

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 18. The albumin fusion protein of any one of claims 1-12, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

 19. The albumin fusion protein of any one of claims 1-12, wherein the albumin fusion protein further comprises a secretion leader sequence.

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 20. A composition comprising the albumin fusion protein of any one of claims 1-12 and a pharmaceutically acceptable carrier.

21. A kit comprising the composition of claim 20.

22. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-12.

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23. The method of claim 22, wherein the disease or disorder comprises indication:Y.

24. A method of treating a patient with a disease or disorder that is modulated by
10 Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-12.

25. The method of claim 24, wherein the disease or disorder is indication:Y.

15 26. A method of extending the shelf life of Therapeutic protein:X, or fragment or variant thereof, comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin, or fragment or variant thereof, sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

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27. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-12.

28. A vector comprising the nucleic acid molecule of claim 27.

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29. A host cell comprising the nucleic acid molecule of claim 28.

30. An albumin fusion protein comprising a member selected from the group

consisting of:

(a) an IL-2 and albumin comprising the amino acid sequence of SEQ ID NO:18;

(b) an IL-2 and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity;

(c) an IL-2 and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity, and further wherein said albumin activity is the ability to prolong the shelf life of the IL-2 compared to the shelf-life of the IL-2 in an unfused state;

(d) an IL-2 and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity, and further wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18;

(e) a fragment or variant of an IL-2 and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has T cell proliferative activity or T cell activation activity;

(f) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the IL-2, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin;

(g) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the IL-2, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin;

(h) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the IL-2, or fragment or variant thereof, is fused to the N-terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin;

(i) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), which comprises the IL-2, or fragment or variant thereof, and a

Therapeutic protein:X, or fragment or variant thereof, wherein said IL-2, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof;

5 (j) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (i), wherein the IL-2, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker; and

(k) an IL-2, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (j), wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

10 and further wherein R1 is IL-2, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or a fragment or variant of albumin.

31. The albumin fusion protein of claim 30, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the IL-2, or fragment or variant thereof, in an unfused state.

32. The albumin fusion protein of claim 30, wherein the in vitro T cell proliferative activity or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro T cell proliferative activity or T cell activation activity of the IL-2, or fragment or variant thereof, in an unfused state.

33. The albumin fusion protein of claim 30, wherein the in vivo T cell proliferative activity or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo T cell proliferative activity or T cell activation activity of the IL-2, or fragment or variant thereof, in an unfused state.

34. An albumin fusion protein comprising an IL-2, or fragment or variant thereof, inserted into an albumin, or fragment or variant thereof, comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

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35. An albumin fusion protein comprising an IL-2, or fragment or variant thereof, inserted into an albumin, or fragment or variant thereof, comprising an amino acid sequence selected from the group consisting of:

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- (a) amino acids 54 to 61 of SEQ ID NO:18;
- (b) amino acids 76 to 89 of SEQ ID NO:18;
- (c) amino acids 92 to 100 of SEQ ID NO:18;
- (d) amino acids 170 to 176 of SEQ ID NO:18;
- (e) amino acids 247 to 252 of SEQ ID NO:18;
- (f) amino acids 266 to 277 of SEQ ID NO:18;
- (g) amino acids 280 to 288 of SEQ ID NO:18;
- (h) amino acids 362 to 368 of SEQ ID NO:18;
- (i) amino acids 439 to 447 of SEQ ID NO:18;
- (j) amino acids 462 to 475 of SEQ ID NO:18;
- (k) amino acids 478 to 486 of SEQ ID NO:18; and
- (l) amino acids 560 to 566 of SEQ ID NO:18.

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36. The albumin fusion protein of claim 34, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the IL-2, or fragment or variant thereof, as compared to the shelf-life of the IL-2, or fragment or variant thereof, in an unfused state.

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37. The albumin fusion protein of claim 35, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the IL-2, or fragment or

variant thereof, as compared to the shelf-life of the IL-2, or fragment or variant thereof, in an unfused state.

38. The albumin fusion protein of claim 34, wherein said albumin fusion protein
5 comprises a portion of albumin sufficient to prolong the in vitro T cell proliferative activity
or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin as
compared to the in vitro T cell proliferative activity or T cell activation activity of the IL-2,
or fragment or variant thereof, in an unfused state.

10 39. The albumin fusion protein of claim 35, wherein said albumin fusion protein
comprises a portion of albumin sufficient to prolong the in vitro T cell proliferative activity
or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin as
compared to the in vitro T cell proliferative activity or T cell activation activity of the IL-2,
or fragment or variant thereof, in an unfused state.

15 40. The albumin fusion protein of claim 34 wherein said albumin fusion protein
comprises a portion of albumin sufficient to prolong the in vivo T cell proliferative activity
or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin
compared to the in vivo T cell proliferative activity or T cell activation activity of the IL-2,
20 or fragment or variant thereof, in an unfused state.

41. The albumin fusion protein of claim 35 wherein said albumin fusion protein
comprises a portion of albumin sufficient to prolong the in vivo T cell proliferative activity
or T cell activation activity of the IL-2, or fragment or variant thereof, fused to albumin
25 compared to the in vivo T cell proliferative activity or T cell activation activity of the IL-2,
or fragment or variant thereof, in an unfused state.

42. The albumin fusion protein of any one of claims 30-41, which is non-

glycosylated.

43. The albumin fusion protein of any one of claims 30-41, which is expressed in yeast.

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44. The albumin fusion protein of claim 43, wherein the yeast is glycosylation deficient.

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45. The albumin fusion protein of claim 43 wherein the yeast is glycosylation and protease deficient.

46. The albumin fusion protein of any one of claims 30-41, which is expressed by a mammalian cell.

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47. The albumin fusion protein of any one of claims 30-41, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

48. The albumin fusion protein of any one of claims 30-41, wherein the albumin fusion protein further comprises a secretion leader sequence.

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49. A composition comprising the albumin fusion protein of any one of claims 30-41 and a pharmaceutically acceptable carrier.

50. A kit comprising the composition of claim 49.

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51. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 30-41.

52. The method of claim 51, wherein the disease or disorder comprises a member selected from the group consisting of: metastatic renal cell carcinoma; metastatic melanoma; malignant melanoma; renal cell carcinoma; HIV infection; inflammatory bowel disorder; Kaposi's sarcoma; leukaemia; multiple sclerosis; rheumatoid arthritis; transplant rejection; type 1 diabetes mellitus; lung cancer; acute myeloid leukaemia; hepatitis C; non-hodgkin's lymphoma; and ovarian cancer.

53. A method of treating a patient with a disease or disorder that is modulated by IL-2, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 30-41.

54. The method of claim 53, wherein the disease or disorder comprises a member selected from the group consisting of: metastatic renal cell carcinoma; metastatic melanoma; malignant melanoma; renal cell carcinoma; HIV infection; inflammatory bowel disorder; Kaposi's sarcoma; leukaemia; multiple sclerosis; rheumatoid arthritis; transplant rejection; type 1 diabetes mellitus; lung cancer; acute myeloid leukaemia; hepatitis C; non-hodgkin's lymphoma; and ovarian cancer.

55. A method of extending the shelf life of IL-2, or fragment or variant thereof, comprising the step of fusing the IL-2, or fragment or variant thereof, to albumin, or fragment or variant thereof, sufficient to extend the shelf-life of the IL-2, or fragment or variant thereof, compared to the shelf-life of the IL-2, or fragment or variant thereof, in an unfused state.

56. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 30-41.

57. A vector comprising the nucleic acid molecule of claim 56.

58. A host cell comprising the nucleic acid molecule of claim 57.

59. An albumin fusion protein comprising albumin, or a fragment or variant thereof, and a protein selected from the group consisting of:

- (a) calcitonin;
- (b) growth hormone releasing factor;
- (c) IL-2 fusion protein;
- (d) insulin-like growth factor-1;
- (e) interferon beta; and
- (f) parathyroid hormone.